Date of Approval: March 24, 2016

FREEDOM OF INFORMATION SUMMARY

ORIGINAL NEW ANIMAL DRUG APPLICATION

NADA 141-460

AIVLOSIN 17%

Tylvalosin Type A Medicated Article

Swine

Control of porcine proliferative enteropathy (PPE) associated with *Lawsonia intracellularis* infection in groups of swine in buildings experiencing an outbreak of PPE

Sponsored by:

ECO LLC

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I. GENERAL INFORMATION

A. File Number

NADA 141-460

B. Sponsor

ECO LLC
344 Nassau St.
Princeton, NJ 08540

Drug Labeler Code: 066916

C. Proprietary Name

AIVLOSIN 17%

D. Established Name

Tylvalosin Type A medicated article

E. Pharmacological Category

Antimicrobial

F. Dosage Form

Type A medicated article

G. Amount of Active Ingredient

Tylvalosin 17% w/w (77.12 g tylvalosin/lb, equivalent to tylvalosin tartrate 19.4% w/w)

H. How Supplied

50 lb (22.7 kg) bag

I. Dispensing Status

Veterinary Feed Directive (VFD)

J. Dosage Regimen

Feed at an inclusion rate of 38.6 grams tylvalosin per ton of Type C medicated feed (42.5 ppm) as the sole ration for 14 consecutive days.

K. Route of Administration

Oral in feed

L. Species/Class

Swine

M. Indication

Control of porcine proliferative enteropathy (PPE) associated with *Lawsonia intracellularis* infection in groups of swine in buildings experiencing an outbreak of PPE.

II. EFFECTIVENESS

A. Dosage Characterization

A dose determination study was conducted to evaluate the effectiveness of 0, 42.5, and 85 ppm of tylvalosin base[1] in feed, provided as the sole ration for 10 days, for the control of PPE associated with *Lawsonia intracellularis* infection using an experimentally induced-challenge model.

One hundred and forty-four (144) growing pigs of both sexes, aged 4-5 weeks, weighing approximately 8-15 kg, and sourced from PPE-free high health farms, were enrolled in the study. A randomized complete block design was used with 6 single sex pigs per pen and 8 pens per treatment group with equal numbers of males and females. Pigs were individually infected by intra-esophageal gavage with an intestinal mucosal homogenate prepared from the affected intestine of a recent North American case of PPE. When at least 15% of study pigs were observed to be clinically affected with PPE based on fecal scores, the 10 day treatments were started (designated as first day of medication or DM). Pigs were fed treatment feed for 10 days (DM+10), then the treatment feed was removed and replaced with non-medicated feed until DM+21. Pigs were necropsied following death or removal from the study or at the end of the study (DM+22). Clinical scores (pig demeanor score, abdominal appearance score, and fecal score), mortality, gross PPE lesion scores, average daily weight gain (ADG), and average daily feed intake were measured during the study.

When given over a 10-day treatment period, the 42.5 and 85 ppm tylvalosin inclusion rates were effective for the control of PPE associated with *Lawsonia intracellularis* infection using an experimentally induced-challenge model. Thirteen pigs died or were euthanized due to PPE: eleven (22.9%) in the 0 ppm group and two (4.3%) in the 42.5 ppm group. Pig demeanor score, abdominal appearance score, fecal score, ADG, and feed efficiency of the pigs in both of the treated groups (42.5 and 85 ppm tylvalosin) were significantly improved ($P<0.05$) compared to the 0 ppm group. The gross PPE lesion scores of the pigs in the 85 ppm group, but not the 42.5 ppm group, were significantly improved compared to the 0 ppm group. The 42.5 ppm tylvalosin inclusion rate was selected for evaluation over a 14-day treatment period in dose confirmation studies using the same challenge model.

B. Substantial Evidence

The effectiveness of tylvalosin, fed at 38.6 grams tylvalosin per ton (42.5 ppm) in Type C medicated feed as the sole ration for 14 consecutive days, for the control of PPE associated with *Lawsonia intracellularis* infection was demonstrated in two adequate and well-controlled dose confirmation studies using an experimentally induced-challenge model. Both studies followed a similar protocol. Studies were

[1] Tylvalosin provided as the tartrate salt

conducted at two different locations in North America and the data from each study were analyzed independently.

1. Study Title: "Determination of the efficacy of Aivlosin® (Tylvalosin) 17% Type A Medicated Article (Premix) in feed of pigs for the control of porcine proliferative enteropathy (PPE, ileitis) in pigs experimentally infected with *Lawsonia intracellularis* (Dose Confirmation Study)." Study Nos. EFF.US.070142 and EFF.US.090170.

2. Study Dates (In-Life Phase):

   EFF.US.070142 – July to August 2008

   EFF.US.090170 - February to April 2009

3. Study Locations:

   EFF.US.070142 - Burford, ON, Canada

   EFF.US.090170 - Rice, MN

4. Study Design:

   a. Objective: To evaluate the effectiveness of tylvalosin Type A medicated article for the control of PPE in pigs with experimentally induced *Lawsonia intracellularis* infections. All personnel involved in the clinical and postmortem assessments were masked to treatments.

   b. Study Animals: A total of 144 healthy commercial cross-bred pigs of both sexes and weighing between 8 and 14 kg were enrolled at each site. Each study obtained pigs from separate high herd health sources. Pigs were approximately 5 to 6 weeks of age at the time of challenge administration. Pigs were confirmed to be free of infection from PPE, Porcine Reproductive and Respiratory Syndrome (PRRS), *Mycoplasma hyopneumoniae*, *Actinobacillus pleuropneumoniae* (APP), transmissible gastroenteritis (TGE), atrophic rhinitis, and mange based on serology and/or clinical observation. PPE-free status was confirmed by fecal polymerase chain reaction (PCR) and serological immunoperoxidase monolayer assay (IPMA) testing on two randomly pre-selected pigs per pen at each site on or soon after arrival. These tests confirmed that the pigs had not been exposed to *L. intracellularis* prior to arrival at each study site.

      Following acclimation, each pig was administered between 10^8 and 10^9 *L. intracellularis* organisms by esophageal gavage. The challenge material was a mucosal homogenate prepared from the affected intestine of a recent North American case of PPE. The original source of the *L. intracellularis* infection for Study EFF.US.070142 was from the intestine of a field case of porcine hemorrhagic enteropathy (PHE) in a finishing pig in 2005. The original source of the *L. intracellularis* infection for Study EFF.US.090170 was from the intestine of a field case of PHE in 2007.

      Samples of gastrointestinal tissue for *L. intracellularis* immunohistochemistry (IHC) were collected after challenge from all pigs that died or were euthanized during the study or at the end of the study. Fecal samples for *L. intracellularis* PCR analysis and blood samples for

serum *L. intracellularis* IPMA were collected from two randomly pre-selected pigs per pen in each study at the end of the in-life phase of the study. These tests confirmed the successful establishment of the *L. intracellularis* challenge infection.

c. Treatment Groups: A randomized complete block design was used in both studies. In each study, within each gender, pigs were weighed, rank ordered, and assigned in groups of 12 pigs to six blocks for a total of 12 blocks. The 12 pigs in each block were randomly assigned to one of two pens, with each pen containing six pigs. The pen was the experimental unit with 12 replicates per treatment group in each study for a total of 72 pigs per treatment in each study.

   Treatments were assigned at random to each pen within each block. Treatments were either an unmedicated control feed or AIVLOSIN 17% (tylvalosin Type A medicated article) incorporated in feed to provide 42.5 ppm tylvalosin.

d. Test Article Administration: In each study, treatment was started when at least 15% of the pigs had fecal scores of ≥1 (semi-solid, not formed). The start of the treatment period was designated as DM (first day of medication). Pigs received their assigned treatment group feed as the sole ration for 14 consecutive days. All pigs received unmedicated feed from arrival to immediately prior to the start of treatment (DM) and from the end of treatment (DM+14) to the end of the study (DM+21).

e. Measurements and Observations: Pigs were observed once daily from DM to DM+21. Health observations included general condition, any abnormal clinical signs, and mortalities.

   The primary variables for determining effectiveness were clinical scores (pig demeanor score, abdominal appearance score, and fecal score), gross PPE lesion scores at necropsy, ADG, and mortality.

   Pig demeanor score was recorded daily from DM to DM+21 and evaluated as follows:

   0 = normal
   1 = slightly to moderately depressed, listless, will stand
   2 = severely depressed; may or may not be recumbent

   Abdominal appearance score was recorded daily from DM to DM+21 and evaluated as follows:

   0 = normal
   1 = moderately gaunt
   2 = severely gaunt

   Fecal score was recorded daily from DM to DM+21 and evaluated as follows:

   0 = no diarrhea
   1 = semi-solid, not formed
   2 = watery stool, <50% water, runs through the floor slats
   3 = profuse projectile diarrhea, >50% water

For the clinical scores (abdominal appearance, demeanor, and fecal score), data analysis was based on the total number of days on which any pig had an abnormal (non-zero) score as a percentage of total pig days, and presented as a mean of the pens in each group for the given time period.

Pigs that died or were euthanized during the study and all pigs remaining at the end of the study (DM+21) were necropsied. Gross PPE lesion score was recorded at necropsy and evaluated as follows:

0 = normal
1 = mild porcine intestinal adenomatosis (PIA), edema, and hyperemia
2 = moderate PIA edema/hyperemia/reticulated serosa and mucosa
3 = severe PIA edema/hyperemia/reticulated serosa and mucosa/gross thickening of the mucosa/blood or fibrin/necrotic enteritis.

The jejunum, ileum, cecum, and colon were scored for all pigs, including pigs that died or were removed during the study. A pig mean lesion score was calculated as the mean of the jejunum, ileum, cecum, and colon scores. A pen mean lesion score was calculated as the mean of the pig mean lesion scores for all pigs in a pen. The pen mean lesion score was analyzed.

Individual live weights were recorded on the first day of medication (DM), last day of medication (DM+14), and at necropsy on DM+21 or upon death or removal from the study to calculate ADG in individual pigs. ADG was calculated and evaluated on a pen basis from DM through DM+21.

Evaluation of mortality due to PPE in each test group included pigs that died or were euthanized during the study and which had lesions typical of PPE at necropsy.

f. Basis for Conclusion of Effectiveness: At least one of the following had to be met in each study in order to demonstrate effectiveness:

At least two of the three clinical scores (pig demeanor score, abdominal appearance score, and fecal score) in the period DM+15 to DM+21 were statistically significantly ($P \leq 0.05$) improved in the tylvalosin-treated group compared to the control group; AND mortality from PPE was not statistically significantly higher ($P \leq 0.05$) in the tylvalosin-treated group compared to the control group.

OR

Intestinal lesion scores were statistically significantly improved ($P \leq 0.05$) in the tylvalosin-treated group compared to the control group; AND ADG was numerically improved in the tylvalosin-treated group compared to the control group; AND mortality from PPE was not statistically significantly higher ($P \leq 0.05$) in the tylvalosin-treated group compared to the control group.

g. Statistical Methods: Each study was analyzed separately. For each of the clinical variables (pig demeanor score, abdominal appearance score, fecal score), the proportion of pig-days with abnormal scores (score of 1 or greater) was calculated for each pen for DM+15 through DM+21. The

arcsine of the square root of the pen proportion was analyzed with a linear mixed model. Fixed effects were treatment, period, and treatment by period interaction. Random effects were block and block by treatment interaction. Akaike's Information Criterion was used in the selection of an appropriate covariance structure for the repeated measures. Significance of the comparison between the tylvalosin-treated and control groups was assessed within each of the time periods using model-based two-sided t tests.

A mean lesion score across the jejunum, ileum, cecum, and colon was calculated for each pig. The mean of the pig mean scores was calculated for each pen and analyzed with a linear mixed model with a fixed effect for treatment and a random effect for block. The comparison of the tylvalosin-treated and control groups were tested using a model-based two-sided t test. ADG was calculated for DM through DM+21 for each pen and analyzed using the same model and procedure as that for lesion scores. Mortality was analyzed with Fisher's Exact Test using individual observations pooled across pens. All testing was done at the 0.05 level of significance.

5. Results:

   a. Study EFF.US.070142

      Treatment with tylvalosin in feed was initiated on Study Day 6 (DM) when 26.4% of the pigs had a fecal score of ≥1 (semi-solid feces) following challenge with *L. intracellularis*. Of the 144 pigs enrolled in the study, one pig from the control group was removed before the start of medication and was not included in the statistical analysis. In addition, a total of five pigs, four in the control group and one in the tylvalosin-treated group, were removed for humane reasons, and were not included in the analysis of mortality or lesion scores. Therefore, the clinical score and ADG analyses included 143 pigs (71 in the control group and 72 in the tylvalosin-treated group), and the lesion score and mortality analyses included 138 pigs (67 in the control group and 71 in the tylvalosin-treated group).

      Excluding the five pigs (four in the control group and one in the tylvalosin-treated group) that were not included in the analysis, nine pigs (7 of 67 pigs [10.4%] in the control group and 2 of 71 pigs [2.8%] in the tylvalosin-treated group) died or were euthanized as a result of PPE infection after the start of treatment. Pig demeanor score and abdominal appearance score were numerically improved, and fecal score was significantly different ($P \leq 0.05$) and improved in the tylvalosin-treated group compared to the control group. Mortality was not significantly different in the tylvalosin-treated group compared to the control group. PPE lesion scores were significantly different ($P \leq 0.05$) and improved in the tylvalosin-treated group compared to the control group. ADG was numerically improved in the tylvalosin-treated group compared with the control group. The results are summarized in Table 1.

Table 1. Summary of effectiveness decision variables, Study EFF.US.070142.

	Control Treatment Group	Tylvalosin Treatment Group
Inclusion Rate (ppm tylvalosin in feed)	0	42.5
Clinical Scores:		
Pen Abnormal Demeanor Score (%)	16.2	9.0 (P=0.0977)
Pen Abnormal Abdominal Appearance Score (%)	32.3	17.0 (P=0.0614)
Pen Abnormal Fecal Score (%)	67.7	39.9 (P<0.0001)
PPE Mortality (%)	10.4	2.8 (P=0.0901)
Pen Mean Lesion Score	0.88	0.66 (P=0.0290)
Pen ADG (kg/pig/day)	0.18	0.34

b. Study EFF.US.090170:

Treatment with tylvalosin in feed was initiated on Study Day 7 (DM) when 16.8% of the pigs had a minimum fecal score of 1 (semi-solid feces) following challenge with *L. intracellularis*. Of the 144 pigs enrolled in the study, one pig in the control group died before challenge and was not replaced. One additional pig in the control group was removed and euthanized before the start of treatment. Therefore, the data analysis included 142 pigs (70 in the control group and 72 in the tylvalosin-treated group).

One pig in the control group died as a result of PPE infection after DM. There were no deaths or removals in the tylvalosin-treated group. Abdominal appearance scores and fecal scores were significantly different ($P \leq 0.05$) and improved in the tylvalosin-treated group compared to the control group. Mortality was not significantly different in the tylvalosin-treated group compared to the control group. PPE lesion scores were significantly different ($P \leq 0.05$) and improved in the tylvalosin-treated group compared to the control group. ADG was numerically improved in the tylvalosin-treated group compared with the control group. The results are summarized in Table 2.

Table 2. Summary of effectiveness decision variables, Study EFF.US.090170.

	Control Treatment Group	Tylvalosin Treatment Group
Inclusion Rate (ppm tylvalosin in feed)	0	42.5
Clinical Scores:		
Pen Abnormal Demeanor Score (%)	5.2	1.4 (P=0.0808)
Pen Abnormal Abdominal Appearance Score (%)	37.2	13.3 (P=0.0038)
Pen Abnormal Fecal Score (%)	51.4	14.7 (P<0.0001)
PPE Mortality (%)	1.4	0.0 (P=0.4930)
Pen Mean Lesion Score	0.61	0.29 (P=0.0221)
Pen ADG (kg/pig/day)	0.61	0.64

6. Adverse Reactions: No adverse reactions attributable to the test article were reported in either study.

7. Conclusion: The results of these two studies demonstrate that AIVLOSIN 17% (tylvalosin Type A medicated article) is effective for the control of PPE associated with *Lawsonia intracellularis* in swine when administered in Type C medicated feed at an inclusion rate of 38.6 grams tylvalosin per ton (42.5 ppm) as the sole ration for 14 consecutive days.

III. TARGET ANIMAL SAFETY

A. Margin of Safety Study

1. Title: Study Number TAS.UK.120273: "Margin of Safety Study of Aivlosin® (17% tylvalosin) Type A Medicated Article (Aivlosin® 17% Premix) in Pigs." September 2012 to November 2012.

2. Study Location: Tranent, Edinburgh, United Kingdom

3. Study Design:

   a. Objective: To provide margin of safety information for tylvalosin Type A medicated article at 0, 38.6, 116, and 193 grams of tylvalosin per ton (0x, 1x, 3x, and 5x the labeled inclusion rate [0, 42.5, 127.5, and 212.5 ppm tylvalosin], respectively) for 42 consecutive days (3 times the labeled duration of treatment).

   b. Study Animals: Thirty-two weaned Landrace/Large White cross pigs (16 non-castrated males and 16 females), aged 4 to 8 weeks, and weighing 9.7 to 17.2 kg on arrival, were used for the study. Animals were acclimated for at least 23 days prior to the treatment period. Animals were individually housed in identically-sized pens. All animals were offered an *ad libitum* amount of commercially available, non-medicated feed. Water was offered to each animal via an individual drinking system.

c. Treatment Groups: Pigs were randomly assigned to one of four treatment groups blocked by gender (Table 3) as well as randomly assigned to one of two equally-sized subgroups on Study Day -8/-9 (for subgroups A and B, respectively).

Table 3. Margin of Safety Study Design - Treatment Groups.

Treatment Groups[a]	Tylvalosin Inclusion Rate in Feed	Number of Animals	Duration of Dosing (Consecutive Days)
1 (0X)	0 ppm	4 intact male/ 4 female	42
2 (1X)	42.5 ppm	4 intact male/ 4 female	42
3 (3X)	127.5 ppm	4 intact male/ 4 female	42
4 (5X)	212.5 ppm	4 intact male/ 4 female	42

[a] Multiples of the labeled tylvalosin inclusion rate in feed in parentheses

d. Test and Control Article Administration: The test article was AIVLOSIN 17% (tylvalosin) Type A medicated article, formulated as the intended commercial product. Non-medicated feed was used as the control article. Medicated or non-medicated feed was supplied to each animal *ad libitum* via individual feed systems for 42 consecutive days (Study Days 1 through 42). On Study Day 43 all animals received non-medicated feed.

e. Measurements and Observations: During the 15-day pre-randomization period, study activities for all animals were identical and each activity occurred on the same calendar day(s). At the time of randomization, two equally-sized subgroups were formed on Study Day -8/-9 (subgroups A and B, respectively). Post-randomization, subgroup A and B study activities were identical but staggered by one calendar day.

1) Physical examinations were conducted by a masked veterinarian pre-randomization on Study Days -10 or -11 (subgroup A)/-11 or -12 (subgroup B), and post-randomization on Study Days -3, 2, 16, 30, and 42, and included, but were not limited to, evaluation of: general appearance and behavior; integument; musculoskeletal system; cardiovascular system; respiratory system; gastrointestinal system; urinary system; reproductive system; lymphatic system; nervous system; ocular system; rectal body temperature; heart rate; respiration rate; and capillary refill rate.

2) General health observations were conducted twice daily (AM and PM) for abnormal behavior or signs of ill health from Study Day –23 (subgroup A)/-24 (subgroup B) to necropsy (Study Day 43).

3) Post treatment general health observations were conducted in all treatment groups on Study Day 1 at 2, 4, and 6 hours (± 30 minutes) after initiation of treatment.

4) The consistency of feces, as well as the presence of blood and/or mucous, was recorded pre-randomization on Study Day -11 (subgroup A)/-12 (subgroup B), and post-randomization on Study Days -3, 2, 16, 30, and 42. Fecal samples were collected for occult blood analysis pre-randomization on Study Days -12 or -13 (subgroup A)/-13 or -14 (subgroup B), and post-randomization on Study Days -5, 4, 22, and 40.

5) Feed and water consumption were measured daily from pre-randomization on Study Day -10 (subgroup A)/-11 (subgroup B) until necropsy (Study Day 43).

6) Body weights were recorded pre-randomization on Study Days -10 (subgroup A)/-11 (subgroup B), and post-randomization on Study Days -3, 2, 16, 30, 42, and prior to necropsy on Study Day 43.

7) Blood samples for hematology, coagulation, and clinical chemistry analysis were collected pre-randomization on Study Days -10 or -11 (subgroup A)/-11 or -12 (subgroup B), and post-randomization on Study Days -3, 2, 16, 30, and 42.

8) Samples for urinalysis and urine microscopy were collected pre-randomization on Study Days -12 or -13 (subgroup A)/-13 or -14 (subgroup B), and post-randomization on Study Days -5, 4, 22, and 40.

9) Gross pathology, selected tissue samples for histopathology, and organ weights were evaluated at necropsy on Study Day 43.

4. Statistical Analysis: Organ weights were analyzed using a mixed model analysis of covariance, with terminal body weight (covariate), sex, treatment, and treatment by sex interactions as fixed effects. Weights of sex organs were analyzed using terminal body weight (covariate) and treatment as fixed effects.

   Repeated measures analysis of variance was used for variables measured at multiple times: hematology, coagulation, and clinical chemistry data; urinalysis data; body weights; and feed and water consumption. The model included a pre-treatment baseline value as a covariate; treatment, time, and gender and their two and three-way interactions as fixed effects; and animal nested in treatment group as a random effect. The covariance structure was selected based on the lowest Akaike Information Criterion value. Contrasts to compare treatments to control were selected based on the significance of the treatment and treatment interaction effects. Contrast tests were evaluated at the 0.10 level of significance.

5. Results:

   a. Mortality: All pigs survived to the scheduled necropsy.

   b. Body weight: There were no statistically significant or clinically relevant differences between the body weights of animals in any of the treated groups compared with the control group.

   c. Feed consumption: There were no statistically significant or clinically relevant differences in feed consumption for any of the treated groups

compared with the control group. All treatment groups showed an increase in mean feed consumption over the duration of the treatment period as was expected in growing pigs.

d. Water consumption: All treatment groups showed an increase in mean water consumption over the duration of the treatment period as was expected in growing pigs. Results of the treatment by sex interaction were significant; therefore, pairwise comparisons were performed for each sex separately. The adjusted mean water consumption over all days was statistically significantly lower for females in the 1X group, but not for females in the 3X and 5X groups, compared with females in the 0X (control) group. The adjusted mean water consumption over all days combined was statistically significantly higher for males in the 5X group compared with males in the 0X (control) group. One of four males in the 5X group consumed larger quantities of water for 9 days during the study which influenced the water consumption results for males; however, there were no associated clinical signs, abnormal clinical pathology results, or abnormal findings with gross necropsy and histopathology. These appeared to be incidental observations and not associated with the test article.

e. Physical Examinations and Clinical Findings: All animals remained in good general health for the duration of the study. One animal in the 3X group developed a corneal ulcer on Study Day 26. The ulcer was treated with ocular saline lavage and resolved by Study Day 30. This was not considered related to the test article. A small number of other minor sporadic health abnormalities were recorded in all treatment groups throughout the course of the study, none of which were of clinical concern or considered to be a result of treatment.

f. Physical Examination Variables: There were no statistically significant or clinically relevant changes noted in heart rate, respiratory rate, capillary refill rate, or rectal temperature.

g. Hematology: The treatment main effect was significant for white blood cell (WBC) counts and absolute neutrophil counts; therefore, pairwise comparisons were made between the treatment (1X, 3X, and 5X) groups and the control (0X) group. The WBC and neutrophil counts over all time points combined were statistically significantly lower in each of the treatment groups compared with the control group. One animal from each treatment group had a single time point in which the WBC or neutrophil count decreased marginally below the study reference range. All values returned to within the study reference range before the study ended. These findings were not considered treatment-related based on lack of dose response, and were not considered clinically relevant based on the transient and mild nature of the decreases and observation in only three animals.

h. Coagulation: There were no statistically significant or clinically relevant findings for the coagulation parameters.

i. Clinical Chemistry: Statistically significant differences between the control (0X) group and one or more treatment (1X, 3X, 5X) groups were observed for alkaline phosphatase, sodium, albumin, and glutamate dehydrogenase.

These findings were incidental, sporadic, not dose-related, and the individual values in the tylvalosin-treated group animals remained within or close to the study reference range. As such, they were not considered to be clinically relevant or treatment-related. The treatment group by time interaction term was significant for alanine aminotransferase (ALT); therefore, pairwise comparisons were performed between the treatment groups (1X, 3X, 5X) and the control (0X) group for each time point separately. ALT values were statistically significantly higher in all treatment groups on Days 16, 30, and 42 compared with the control group. Although the ALT in several animals at various time points was marginally increased above the study reference range, these ALT values were acceptably within the range of ALT values observed prior to treatment in all animals and in the control (0X) group throughout the study. These mild increases in ALT may have been related to the test article as the increases showed a trend corresponding to increased dose and/or duration; however, the increases in ALT were not accompanied by clinical signs or gross or microscopic necropsy abnormalities; therefore, they were not considered clinically relevant.

j. Urinalysis: There were no statistically significant or clinically relevant findings for urine specific gravity results. One animal in the 3X group had low specific gravity on Study Day 40; however, it was not correlated to dose, duration, individual water consumption, or individual blood urea nitrogen and creatinine levels. Other urinalysis parameters and microscopic examination of urine sediment were not statistically analyzed, and were unremarkable for all animals.

k. Fecal Consistency: Fecal scores of "semi-solid" were observed sporadically in all treatment groups. In many cases the feces were semi-solid during the pre-treatment phase of the study and continued into the treatment period. The changes in fecal consistency were not considered to be related to treatment.

l. Fecal Cytology: Fecal occult blood was negative for all pigs in the study.

m. Gross Pathology: There were no gross necropsy findings related to treatment.

n. Microscopic Pathology: All findings were typical of spontaneously occurring background pathology in pigs of this age and occurred in all treatment groups. There were no histopathological findings considered related to treatment.

o. Organ Weights: There were no statistically significant differences between the control group and treated groups.

p. Organ Weights as a Percentage of Brain Weights: Mean organ weights expressed as a percentage of brain weight were similar across all treatment groups for all organs with the exception of the testes, which appeared heavier in the 1X group. However, examination of the paired testes organ weights showed that differences were variable within treatment groups, the differences were not statistically significant, and there were no dose-related trends. In addition, there were no abnormal

findings in the testes during gross necropsy or histopathological examination.

6. Conclusion: This study demonstrated that AIVLOSIN 17% (tylvalosin Type A medicated article) is safe when administered at 38.6 grams tylvalosin per ton (42.5 ppm) of Type C medicated feed as the sole ration for 14 consecutive days.

IV. HUMAN FOOD SAFETY

A. Microbial Food Safety (Antimicrobial Resistance)

Microbial food safety information (antimicrobial resistance) for tylvalosin was evaluated using a qualitative risk assessment procedure. The dosage regimen evaluated was 42.5 ppm (38.6 g/ton) tylvalosin in Type C medicated feeds, fed to swine as a sole ration for 14 consecutive days. The indication associated with this dosage regimen is, "For the control of porcine proliferative enteropathy (PPE) associated with *Lawsonia intracellularis* infection in groups of swine in buildings experiencing an outbreak of PPE."

The qualitative risk assessment procedure involved conducting 1) a release assessment to describe the probability that tylvalosin and its use in swine will result in the emergence of macrolide-resistant bacteria or macrolide resistance determinants in treated swine under proposed conditions of use; 2) an exposure assessment to describe the likelihood of human exposure to macrolide-resistant bacteria or macrolide resistance determinants through consumption of edible products from treated swine; and 3) a consequence assessment to describe potential human health consequences arising from exposure to macrolide-resistant bacteria or macrolide resistance determinants by considering the human medical importance of macrolides used in the treatment of human infectious diseases.

It was determined that the risk of development of transferable macrolide resistance elements from this use of tylvalosin in swine is medium. This decision is also supported by National Antimicrobial Resistance Monitoring Program (NARMS) data from animal, retail meat, and human isolates, where little change has been observed in recent years with regard to *Campylobacter* susceptibility to macrolides. Also, changes have not been demonstrated among *Enterococcus* spp., and, due to pre-existing macrolide resistance, this is of less significance.

Macrolides are ranked as critically important drugs in human medicine; therefore, by default, the consequence assessment yields a high ranking. The overall risk estimation is derived to be high. The proposed conditions of use, and restriction of use to only groups of swine in buildings experiencing an outbreak of PPE are compatible with the Agency's risk management strategies associated with a product having an overall risk estimation of high.

Decision Statement

The Agency's integration of the degree of risk derived from the three individual assessments (medium, medium, and high) gave an overall risk estimation of high. The conditions of use are compatible with the Agency's risk management strategies for a Category 1 drug, corresponding to the estimated high risk. The product will be available by veterinary feed directive (VFD) only. Further, post-

approval monitoring may be achieved from testing of surrogate antimicrobials (erythromycin and azithromycin) in the current NARMS program.

B. Impact of Residues on Human Intestinal Flora

The effects of tylvalosin residues on human intestinal flora were assessed through the following approaches.

1. Determination of the need for establishing a microbiological ADI

   a. Step 1: Are residues of tylvalosin and (or) its metabolites microbiologically active against representatives of the human intestinal flora?

      Yes, it has been concluded that tylvalosin and its residues are active against representative human intestinal flora. An *in vitro* study was performed to further confirm the conclusion and to help calculate the activity. A brief summary of the study is provided below.

      Study Title: Activity of acetylisovaleryltylosin and 3-acetyltylosin against bacterial strains representing the normal human intestinal microbiota: determination of minimum inhibitory concentration (MIC).
      Study Number: DWS P1/015/04
      Report Date: January 28, 2005
      Study Location: Shipley, West Yorkshire, United Kingdom
      Study Summary: Susceptibility testing of acetylisovaleryltylosin (tylvalosin) and its metabolite 3-acetyltylosin was performed against 100 bacterial isolates (10 isolates from each of 10 genera) representative of human intestinal flora. The isolates came from feces of healthy, non-medicated human volunteers. The methodology used was the agar dilution method as described in Clinical and Laboratory Standards Institute (CLSI) guidelines performed at a single inoculum level as recommended in CLSI guidelines. ATCC strains of *Bacteroides fragilis* (25285), *Eubacterium lentum* (43055), *Staphylococcus aureus* (29213), and *Enterococcus faecalis* (29212) were used to monitor performance and reproducibility of the testing.
      Results and Conclusions: The *in vitro* activity of the two compounds against representative bacterial groups is summarized in Table 4. Except for *Escherichia coli*, the compounds showed potent and moderate activities against organisms tested. Using a cutoff value of 8 µg/mL, 9 of the 10 groups were included in the calculation of overall activities for each compound based on MIC_{50}s. The mean MIC_{50} values for the susceptible genera were 0.335 µg/mL for acetylisovaleryltylosin and 0.256 µg/mL for 3-acetyltylosin.

   b. Step 2: Do tylvalosin residues enter the human colon?

      Yes, by default, it is concluded that tylvalosin and its metabolites enter the human colon.

c. Step 3: Do tylvalosin residues entering the human colon remain microbiologically active?

Yes, a fraction of tylvalosin residues entering the human colon remain biologically active, as shown by the study data. The study is summarized below.

Table 4. Summary of susceptibility of tylvalosin and its metabolite 3-acetyltylosin (µg/mL)

	Tylvalosin	Tylvalosin	Tylvalosin	3-acetyltylosin	3-acetyltylosin	3-acetyltylosin
	MIC_{50}	MIC_{90}	MIC Range	MIC_{50}	MIC_{90}	MIC Range
Bacteroides fragilis	0.5	2	0.25-2.0	1.0	8	0.5-8
Bacteroides spp.	0.5	4	0.125-4	1.0	4	0.125-32
Bifidobacterium spp.	0.06	0.125	0.06-0.125	0.06	0.5	0.06-0.5
Clostridium spp.	0.25	0.5	0.06-0.5	0.06	0.25	0.06-0.5
Eubacterium spp.	0.5	0.5	0.5-1.0	0.25	0.5	0.25-2
Fusobacterium spp.	4	8	0.5-32	0.5	2	0.125-32
Peptostreptococcus	0.5	0.5	0.125-1.0	0.5	1.0	0.25-4
Enterococcus spp.	1.0	8	1-8	2	8	1-8
E. coli	>128	>128	>128	>128	>128	>128
Lactobacillus spp.	2	>128	0.5->128	4	>128	0.5->128
All isolates	**0.5**	**>128**	**0.06->128**	**0.5**	**>128**	**0.06->128**

Study Title: Effect of fecal binding on antibacterial activity of acetylisovaleryltylosin and 3-acetyltylosin.
Study No. DWS P1/016/04
Report Date: February 23, 2005
Study Location: Shipley, West Yorkshire, United Kingdom
Study Summary: The objective of the study was to determine the effect of fecal binding on the antibacterial activity of acetylisovaleryltylosin and 3-acetyltylosin. Stock solutions of acetylisovaleryltylosin and 3-acetyltylosin were freshly prepared on the day of experiment. Selected concentrations of both compounds (0, 1, 2, 5, 10, 20, 50, and 100 µg/mL) prepared in microbiological culture medium were incubated with increasing concentrations of sterilized human feces (0, 10, 25, and 50% w/v) from

3 separate healthy male and female donors. The mixtures were incubated for 30 mins, 1, 2, 6, or 8 hours. Fecal solids from each mixture were removed by centrifugation at the end of each time period. The supernatants of the mixtures from each test compound/fecal slurry were inoculated with *Enterococcus faecalis* (ATCC 29212) and incubated for 24 hours to assess antibacterial activity of the compound in the supernatants. Growth of the test strain was estimated by turbidity in each of fecal supernatants compared to growth medium without fecal materials. This comparison gave an indication of the unbound drug or metabolite in each preparation.

Results and Conclusions: Concentrations of fecal slurries from 10% to 50% showed apparent effects of solids on binding, and maximum binding was seen with 50% feces. Without exposure to feces, each test compound inhibited *E. faecalis* growth at a concentration of 1 µg/mL. With 50% feces, approximately 90% of binding occurred soon after incubation began and maximum binding (at least 98%) occurred between 0.5 hours and 6 hours of interaction for 2/3 donors (and the third donor had 98% binding at 0 incubation time). Thus, it was concluded that up to 2% of the fraction of tylvalosin residues in the fecal environment remain biologically active.

d. Step 4: Determination if there is any scientific justification to eliminate testing for either one or both endpoints of concern:

- **Colonization barrier disruption**

  It was determined that a microbiological ADI (mADI) is to be determined based on this endpoint.

- **Changes in resistant populations**

  Enterococcus spp. was used as an index intestinal bacterium for assessing whether there is scientific justification to eliminate this endpoint. A mADI for resistance is not needed for tylvalosin residues, because macrolides are not used in the treatment of enterococci infections, and decreased activity for macrolides already exists in the enterococci population from humans. It was difficult to determine a NOEAC or NOAEL for this purpose; therefore, it was concluded that a mADI is not to be determined for the endpoint of changes in resistant populations among human intestinal flora.

2. Determination of the final Microbiological ADI

a. Determination of the fraction of oral dose available to microorganisms

From the fecal binding study described in Step 3 (above), it was determined that the fraction of the oral dose of tylvalosin residues available to microorganisms is 2%.

b. Determination of the Microbiological ADI using MIC_{calc}

The mADI was determined based on the following formula with MIC_{calc}.

$$ADI(\mu g/kg\,BW/day) = \frac{MIC_{calc}\ X\ Mass\,of\,colon\,content}{Fraction\,of\,dose\,available\ X\ 60\,kg}$$

MIC_{calc}s of tylvalosin and 3-acetyltylosin derived from the MIC study (Study # DWS P1/015/04) were 0.335 µg/mL and 0.256 µg/mL, respectively. The smaller of the two was used as the MIC_{calc}. The fraction of dose available was 2%. Using the formula, the following mADI of tylvalosin is determined:

mADI = 0.26 x 220/0.02 x 60 = 47.7 µg/kg bw/day

Determination of Microbiological ADI:

The mADI for tylvalosin is 47.7 µg/kg bw/day, or 2.86 mg/person/day.

C. Toxicology

Reassessment of the toxicological acceptable daily intake (ADI) was not needed for this NADA approval. The FOI summary for the original approval of NADA 141-336, dated July 6, 2012, contains a summary of all toxicology studies and information.

D. Establishment of the Final ADI

The final ADI is the microbiological ADI (47.7 µg/kg bw/day) for total residues of tylvalosin. The codified ADI is listed under 21 CFR 556.748.

E. Safe Concentrations for Total Residues in Edible Tissues

The safe concentrations of total residues of tylvalosin in each edible tissue of swine are 2.9 ppm for muscle, 8.6 ppm for liver, 17.3 ppm for kidney, and 17.3 ppm for fat.

F. Residue Chemistry

1. Summary of Residue Chemistry Studies

a. Total Residue and Metabolism Studies

A total residue and metabolism study (Inveresk Report Number 16922) was conducted for the use of AIVLOSIN 17% (tylvalosin Type A medicated article) in swine. The FOI Summary for the original approval of NADA 141-336, dated July 6, 2012, contains a summary of the study. The results of the study support a zero withdrawal assignment for the use.

b. Comparative Metabolism Study

The FOI Summary for the original approval of NADA 141-336, dated July 6, 2012, contains a summary of comparative metabolism studies for the use of AIVLOSIN 17% (tylvalosin Type A medicated article) in swine.

c. Study to Establish Withdrawal Period

Tissue Residue Depletion Study

Study Number and Title
Report No. 009-01065: Determination of Tylvalosin Residues in Liver of Swine Receiving Aivlosin Type A Medicated Article (17% w/w Tylvalosin) for 14 Consecutive Days in Feed Containing 42.5 ppm Tylvalosin

Study Dates
December 2009 to February 2010

Study Locations
In life: Las Cruces, NM
Analytical: Plainsboro, NJ

Test Material and Dosage Form
AIVLOSIN 17% Type A medicated article (17% w/w tylvalosin) administered in feed.

Test Animals
Twenty-two large white/Duroc cross-bred pigs (11 barrows and 11 gilts), approximately 6 months old, weighing 252 – 296 lbs at study commencement.

Dose and Route of Administration
Tylvalosin was administered to target a dose of 42.5 ppm tylvalosin in feed for 14 consecutive days. Incurred tylvalosin concentration was 39.5 ppm in feed.

Tissue Sample Collection and Analysis
Animals were slaughtered at 0, 12 and 24 hours after the last treatment. Tylvalosin concentrations in liver samples were determined for each animal using a validated HPLC-MS/MS procedure.

Results
The highest concentration of parent tylvalosin residue in swine liver at 0 hours after the last treatment was 81 ng/g. All the concentrations of parent tylvalosin residue in swine liver at 12 hours (time point for supporting a zero withdrawal assignment) and 24 hours after the last treatment were below the Limit of Quantitation (LOQ) of the method (50 ng/g).

Conclusion
The study results are consistent with assigning a zero withdrawal for the use of AIVLOSIN 17% (tylvalosin) Type A medicated article in swine administered at an inclusion rate of 42.5 ppm in Type C medicated feed as the sole ration for 14 consecutive days.

2. Target Tissue and Marker Residue

It is not necessary to assign a target tissue or a marker residue for tylvalosin residues in swine.

3. Tolerance

   A tolerance for tylvalosin in swine is not required.

4. Withdrawal Period

   No withdrawal period is required (*i.e.*, zero withdrawal).

G. Analytical Method for Residues

A regulatory analytical method for monitoring tylvalosin residues in swine is not required.

V. USER SAFETY

Not for use in humans. Keep out of reach of children.

May cause skin irritation. Tylvalosin has been shown to cause hypersensitivity reactions in laboratory animals.

People with known hypersensitivity to tylvalosin should avoid contact with this product. In case of accidental ingestion or inhalation, seek medical attention. When handling Aivlosin® 17% Type A Medicated Article and preparing medicated feeds, avoid direct contact with the eyes and skin. Wear a dust mask, coveralls and impervious gloves when mixing and handling this product. Eye protection is recommended. In case of accidental eye exposure, wash eyes immediately with water and seek medical attention. If wearing contact lenses, immediately rinse the eyes first, then remove contact lenses and continue to rinse the eyes thoroughly and seek medical attention.

In case of accidental skin exposure, wash contaminated skin thoroughly.

The Safety Data Sheet contains more detailed occupational safety information.

VI. AGENCY CONCLUSIONS

The data submitted in support of this NADA satisfy the requirements of section 512 of the Federal Food, Drug, and Cosmetic Act (FD&C Act) and 21 CFR part 514. The data demonstrate that AIVLOSIN 17%, when used according to the label, is safe and effective for the control of porcine proliferative enteropathy (PPE) associated with *Lawsonia intracellularis* infection in groups of swine in buildings experiencing an outbreak of PPE. Additionally, data demonstrate that residues in food products derived from species treated with AIVLOSIN 17% will not represent a public health concern when the product is used according to the label.

A. Marketing Status

A valid veterinary feed directive (VFD) is required to dispense this drug. Federal law restricts medicated feed containing this VFD drug to use by or on the order of a licensed veterinarian. Use of feed containing a VFD drug in a manner other than as directed on the labeling (extralabel use) is not permitted. VFDs for tylvalosin shall not be refilled.

The decision to restrict this drug to use by or upon the order of a licensed veterinarian was based on the following factors: (a) adequate directions cannot be written to enable lay persons to appropriately and safely use this product and (b) restricting this drug to use by or upon the order of a licensed veterinarian should help prevent indiscriminate use, which could result in violative tissue residues.

B. Exclusivity

AIVLOSIN 17%, as approved in our approval letter, qualifies for THREE years of marketing exclusivity beginning as of the date of our approval letter. This drug qualifies for exclusivity under section 512(c)(2)(F)(ii) of the FD&C Act because the sponsor submitted an original NADA that contains new studies that demonstrate the safety and effectiveness of AIVLOSIN 17%.

C. Patent Information

For current information on patents, see the Animal Drugs @ FDA database or the Green Book on the FDA CVM internet website.